

We claim:

- 1           1. A method for increasing the production of clavulanic acid in a host comprising the  
2    step of:  
3           increasing the level of N<sup>2</sup>-(2-carboxyethyl)arginine synthase in said host, wherein  
4    said N<sup>2</sup>-(2-carboxyethyl)arginine synthase catalyzes the condensation of L-arginine and D-  
5    glyceraldehyde-3-phosphate, resulting in increased production of clavulanic acid.
- 1           2. The method of claim 1 wherein said step of increasing is performed by gene dosing.
- 1           3. The method of Claim 2 wherein said increasing step is performed by providing said  
2    host with DNA encoding said N<sup>2</sup>-(2-carboxyethyl)arginine synthase.
- 1           4. The method of claim 3 wherein said DNA is in a plasmid.
- 1           5. The method of claim 4 wherein said plasmid is a replicating plasmid.
- 1           6. The method of claim 5 wherein said replicating plasmid is pKC1139/pro-orf2-ter.
- 1           7. The method of claim 3 further comprising integrating said DNA into the  
2    chromosome of said host.
- 1           8. The method of claim 7 wherein said DNA is stably integrated via an integrative  
2    vector selected from the group consisting of pSET152/pro-orf2, pSET152/ermE(XbaI)-orf2  
3    and pSET152/ermE(HindIII)-orf2.

1           9. The method of claim 3 wherein expression of said DNA is under the control of a  
2           constitutive promoter.

1           10. The method of claim 3 wherein said constitutive promoter is *ermE*\*.

1           11. The method of claim 1 wherein said increasing step is performed by adjusting  
2           fermentation conditions and/or providing additives which effect the optimization of  
3           N<sup>2</sup>-(2-carboxyethyl)arginine synthase activity, wherein said optimization results in an  
4           increase in the production of clavulanic acid

1           12. The method of claim 1 wherein said host is *Streptomyces clavuligerus*.

1           13. A method for increasing the production of clavulanic acid in a host comprising the  
2           step of:

3           increasing the availability of precursors for reaction by N<sup>2</sup>-(2-carboxyethyl)arginine  
4           synthase, wherein said step of increasing results in an increase in the production of  
5           clavulanic acid.

1           14. The method of claim 13 wherein said precursors are L-arginine and D-  
2           glyceraldehyde-3-phosphate.

1           15. The method of claim 13 wherein said host is *Streptomyces clavuligerus*.

1           16. The method of claim 13 wherein said increasing step is achieved by adjusting  
2           fermentation conditions and/or providing additives which optimize  
3           N<sup>2</sup>-(2-carboxyethyl)arginine synthase activity.

1 17. A method for increasing the production of N<sup>2</sup>-(2-carboxyethyl)arginine in a host  
2 cell, comprising,

3 enhancing a rate of condensation of L-arginine and D-glyceraldehyde-3-phosphate in  
4 said host cell, wherein said step of enhancing results in an increase in the production of  
5 N<sup>2</sup>-(2-carboxyethyl)arginine in said host cell.

1 18. The method of claim 17 wherein said condensation of L-arginine and D-  
2 glyceraldehyde-3-phosphate is catalyzed by the enzyme N<sup>2</sup>-(2-carboxyethyl)arginine  
3 synthase.

1 19. The method of claim 17 wherein said step of enhancing is carried out by increasing  
2 the copy number of a gene encoding N<sup>2</sup>-(2-carboxyethyl)arginine synthase.

1 20. The method of claim 17 wherein said step of enhancing is carried out by adjusting  
2 fermentation conditions and/or providing additives which optimize  
3 N<sup>2</sup>-(2-carboxyethyl) arginine synthase activity.

1 21. A method for preparing an composition having N<sup>2</sup>-(2-carboxyethyl)arginine  
2 synthase activity, comprising the steps of

3 growing a culture of a host cell capable of synthesizing N<sup>2</sup>-(2-carboxyethyl)arginine  
4 synthase,

5 harvesting and sonicating said culture,

6 removing cellular debris to produce a cellular supernatant,

7 fractionating said supernatant with ammonium sulfate to form a precipitated protein  
8 pellet,

9 resuspending said precipitated protein pellet to form a protein solution, and,

10 chromatographing said protein solution by affinity chromatography to isolate a

11 thiaminepyrophosphate-dependent enzyme having N<sup>2</sup>-(2-carboxyethyl)arginine synthase  
12 activity.

1 22. The method of claim 21 wherein said affinity chromatography is carried out with  
2 an L-arginine agarose affinity column.

1 23. The method of claim 21 wherein said host is *Streptomyces clavuligerus*.

1 24. The method of claim 21 wherein said step of fractionating is carried out with 30%  
2 ammonium sulfate.

1 25. An assay for identifying substrates of the enzyme N<sup>2</sup>-(2-carboxyethyl)arginine  
2 synthase, comprising the steps of

3 incubating a putative substrate with the enzyme N<sup>2</sup>-(2-carboxyethyl)arginine  
4 synthase, thiaminepyrophosphate, and one known substrate of N<sup>2</sup>-(2-carboxyethyl)arginine  
5 synthase, and

6 detecting the presence or absence of a condensation product of the putative substrate  
7 and the known substrate, wherein the presence of a condensation product is a positive result.

1 26. The assay of claim 25 wherein said known substrate is L-arginine.

1 27. The assay of claim 25 wherein said known substrate is D-glyceraldehyde-3-  
2 phosphate.

1 28. A host cell stably transformed with *orf2*.

1 29. The host cell of claim 28, wherein said host cell is *Streptomyces clavuligerus*.

30. A condensation product of two substrates condensed by  
N<sup>2</sup>-(2-carboxyethyl)arginine synthase.